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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Ling et al.	Attorney Docket No. CIBT-P01-119
Serial No: 09/883,848	Art Unit: 1642
Filed: June 18, 2001	Examiner: B. Fetterolf
For: Angiogenesis-Modulating Compositions and Uses	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.131

Sir:

I, Leona Ling hereby declare:

1. I am a named inventor of the pending claims of the patent application identified above and an inventor of the subject matter described in the patent application.

2. Prior to March 30, 2000, the effective filing date of Porter et al. (U.S. Patent No. 6,613,798), I conceived the invention as described and claimed in the subject application in this country as evidenced by the initial observations and experimental plan described in my notebook (attached hereto as Exhibit 1). As summarized in Exhibit 1, based on the expression of the hedgehog receptor patched in the vasculature and in smooth muscle cells, I hypothesized that activation of hedgehog signaling could be used to promote angiogenesis. I recognized that exemplary agents that could be used to activate hedgehog signaling, thereby promoting angiogenesis, include hedgehog proteins and lipophilic modified hedgehog proteins, as well as other agonists of hedgehog signaling. Exhibit 1 demonstrates that I had conceived of methods of using hedgehog agonists to promote angiogenesis. Furthermore, Exhibit 1 demonstrates that I had conceived and articulated specific experiments designed to confirm the effects of hedgehog

signaling on angiogenesis. Accordingly, I had possession of the method of promoting angiogenesis using a hedgehog agonist that promotes hedgehog signaling prior to March 30, 2000.

3. In light of the research plan articulated in Exhibit 1, experiments were conducted under my direction in an outside laboratory in a NAFTA or WTO country. The effect of Sonic hedgehog on angiogenesis was assessed using the corneal plug assay. This assay was specifically enumerated in the research plan, as shown in Exhibit 1. Exhibit 2 depicts the results of an exemplary experiment conducted prior to March 30, 2000, and thus shows reduction to practice of the method of promoting angiogenesis using a hedgehog agonist that promotes hedgehog signaling prior to March 30, 2000. Briefly, Sonic hedgehog protein was tested in a mouse corneal plug assay using ptcLacZ reporter mice. Administration of Sonic hedgehog protein induced angiogenesis in comparison to control corneal plugs. Additionally, administration of Sonic hedgehog protein activated hedgehog signaling, as measured by induction of expression of the hedgehog responsive gene, ptc. These results demonstrated that activation of hedgehog signaling via application of a hedgehog protein could be used to promote angiogenesis. These results further suggested that other hedgehog agonists could similarly be used to promote angiogenesis.

4. I assert that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I also understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 USC 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Leona E Ling

Leona Ling

Dated: 3/21/05

Proposal

L.Ling/M. Sanicola

Initial Results:

1. PtcLacZ animals have shown that ptc is expressed in the adventitial cells of the coronary, aortic and pulmonary arteries of young animals (9 day old). The aortic endothelium and a few cells in the medial SMC layer also showed lacZ staining. These data suggest that adventitial cells, some endothelium and perhaps a subpopulation of SMC or other medial layer cells is responsive to hh in young animals (SS/LL/MS). This distribution of ptc is in line with the mesenchymal expression of ptc in other tissues.

2. PtclacZ day 9 mice also show possible staining of SA or AV node tissue which needs to be confirmed (SS/MM/MS).

3. Volkhard Lindner found expression of Dhh in activated endothelial cells (EC) and Shh in activated SMCs following balloon injury of rat aorta.

4. Hedgehog protein was below the level of detection by immppt of lysates of adult rat aorta. If hh is normally present in adult aorta, it is at low levels.

5. Dhh is expressed in the truncus arteriosus at embryonic day 10.5 dpc and 14 dpc (SS/MS). Ptc is also expressed in the vessels at 14 dpc. Bitgood and McMahon described the expression of Dhh in the endocardium of the AV canal and truncus arteriosus and in endothelial cells of major vessels from 11.5-14.5 dpc.

6. Mark Majesky's lab has made the observation that Shh and Dhh induces rapid mesenchymal transition and SMC differentiation of the PEO (coronary vessel anulage) and decreases proliferation of the PEO cells. Shh is synergistic with TGFb for inducing SMC differentiation of the PEO.

Hypotheses, Background and Further Expts:

1. The expression of Dhh in EC and Shh in SMC which are migrating and proliferating suggest that these hh's may be involved in activating or maintaining the activated phenotype. The results from the Lindner rat vascular injury model and the observation of ptcLacZ expression in a subpopulation of medial (SMC?) in day 9 mice both support the correlation of hedgehog expression with proliferating cells since day 9 vasculature still contains a low number of proliferating SMCs compared to adult vasculature. Thus hh's may be involved in adult vascular remodelling and perhaps angiogenesis.

- Determine Ptc (and hh) expression by *in situ* of en face and crossectional rat vascular injury tissue to see if hedgehog pathway is autocrine or paracrine in activated SMC and EC (VL/SS).
- Determine PtcLacZ expression in 4 month old mice to see if ptc expression diminishes along with proliferation index in adult vasculature (LL).
- Determine if myr-Shh, Ihh or Dhh induces ptc response (RT-PCR), proliferation (3Hthy/BrdU) or migration (scratch/explant/Boyden chamber) of primary EC or SMC *in vitro* (JLY/SS).
- Determine if 5E1 blocks SMC and EC activation during vascular response to injury (TBD).
- Determine if locally delivered (BV or pluronic gel) hh increases SMC or EC activation in normal vessels or following vascular injury (GS?)
- Determine if Dhh or other hh induces angiogenesis (EC) or is synergistic with VEGF or FGF in corneal pocket assay (JI?)
- Determine if hh acts synergistically with VEGF in vasculogenic collateral vessel formation (JI?)

2. Embryonic expression of Dhh in EC or Shh expression in SMC may be important for vasculogenesis and angiogenesis in general or specifically in the PEO/coronary system. Interaction of the PEO cells with the cardiac tissue induces a epithelial to mesenchymal transition. Later EC from the liver primordia migrate to the cardiac surface and interact with these mesenchymal cells to induce SMC differentiation and vasculogenesis.

In other vasculogenic processes, VEGF and perhaps bFGF stabilizes the formation of EC structures which in turn induce recruitment of local mesenchymal cells via PDGF and other unidentified factors including flow-induced factors. Further stabilization of vasculature and remodelling in the embryo is believed to involve TIE/angiopoietin and TGFb1. In addition to vasculogenesis, some vascular beds are formed via angiogenesis of preexisting vessels (brain and kidney). EC and pericytes from preexisting vessels migrate out and form new capillaries which then remodel/mature into larger vessels.

- Determine ptc/LacZ expression during embryonic stages of vasculogenesis and vessel remodelling and growth (SS/MS/MM)
- Determine if ptc/gli are upregulated upon PEO activation by Shh or Dhh (KC)
- Determine the expression of hedgehogs and ptc1 during chick PEO/coronary development by in situ (SS/MS/MM)
- Determine if 5E1/AP.G6 blocks chick PEO/coronary development or general vasculogenesis (MM)
- Determine if 5E1/AP.G6 inhibits vasculogenesis in maternal transfer in mouse (Lwang)
- Determine if overexpression of Dhh in EC during development (TIE-, etc transgenic) induces vascular phenotype (SS/MS)

3. Adventitial location of ptc suggests adventitial fibroblasts may be responsive to hh. Hedgehog may play a role in induction of adventitial myofibroblasts following vascular injury. The induction of adventitial myofibroblasts is believed to contribute to adventitial fibrosis and intimal thickening resulting decreased arterial flow during human restenosis and in the porcine coronary restenosis model. It is postulated that during vascular injury adventitial myofibroblasts as well as medial SMCs are activated to become proliferative and can migrate through the medial SMC layer and contribute to intimal hyperplasia.

- Determine if primary vascular adventitial cells are responsive to myr-Shh, Ihh or Dhh *in vitro*. (JLY/LL)

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ssed & Understood by me, | Date | Invented by [signature] | Date [Redacted]

002/002

Exhibit 1

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Shh induces corneal neovascularization via Ptc1

X-gal staining in Ptc1LacZ mice

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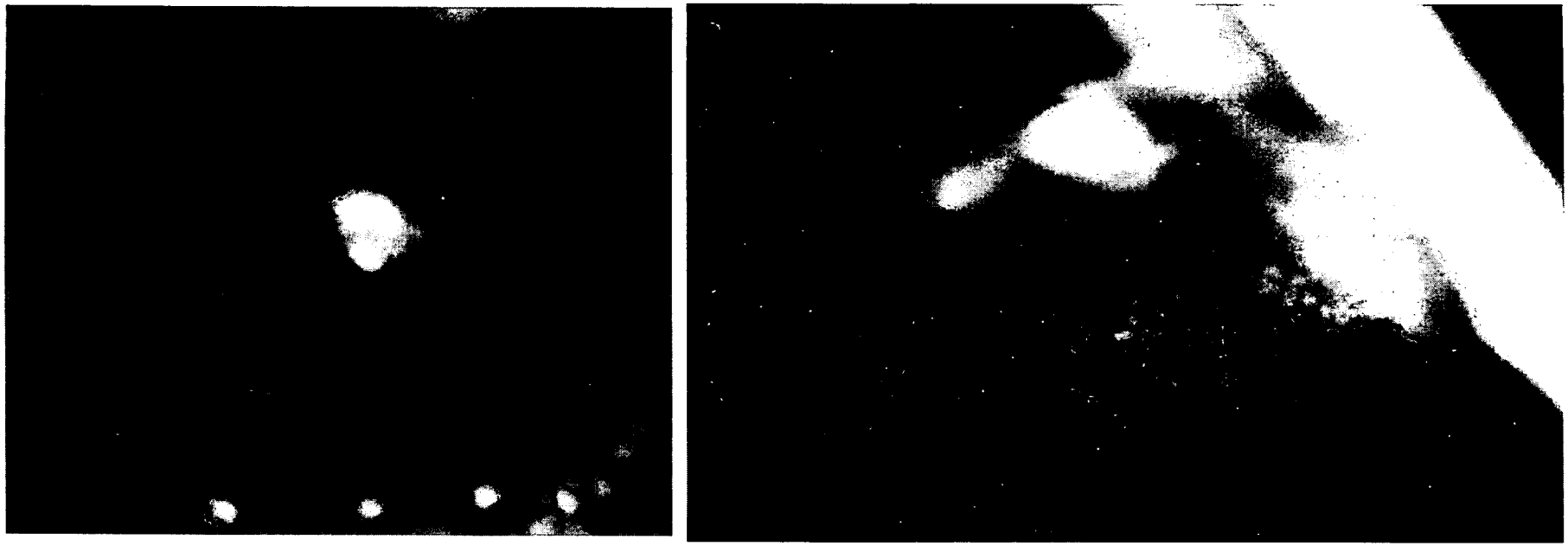

Shh-induced neovascularization in the cornea of a Ptc1LacZ mouse

Exhibit 2

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Application No. (if known): 09/883848 Attorney Docket No.: CIBT-P01-119

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Declaration Under 37 CFR 1.131 (2 pages)
Exhibits 1 and 2 (2 pages)